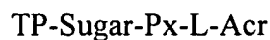


Claims

1. A method of detecting the presence of reverse transcriptase in a sample comprising:
contacting the sample with
an RNA template;
a DNA primer;
one or more deoxynucleotide triphosphates labeled with a detectable moiety and, optionally, one or more deoxynucleotide triphosphates not labeled with a detectable moiety;
incubating said reaction mixture under conditions suitable to generate a molecular structure comprising an extended primer comprising said detectable moiety when reverse transcriptase is present in the sample; and
detecting reverse transcriptase in the sample.
2. The method of claim 1 wherein one of said RNA template, DNA primer, deoxynucleotide triphosphate labeled with a detectable moiety, and deoxynucleotide triphosphate not labeled with a detectable moiety, comprises a capture moiety.
3. The method of claim 1 wherein the detecting step comprises detecting the molecular structure.
4. The method of claim 3 wherein the molecular structure comprises the RNA template, the DNA primer, and one or more deoxynucleotide triphosphates labeled with the detectable moiety and, optionally, one or more deoxynucleotide triphosphates not labeled with the detectable moiety.
5. The method of claim 1 wherein the detectable moiety is an acridinium moiety and the detectable signal is the emission of light.

6. The method of claim 1 further comprising separating the molecular structure from the reaction mixture before generating the detectable signal.
7. The method of claim 1 further comprising that unreacted deoxynucleotide triphosphates are removed from the reaction mixture prior to generating the detectable signal.
8. The method of claim 1 wherein the detectable signal is the emission of light and generating the detectable signal comprises the addition of a dilute acid and hydrogen peroxide to the reaction mixture.
9. The method of claim 1 wherein the RNA template comprises homopolymeric and/or heteropolymeric RNA.
10. The method of claim 1 wherein the deoxynucleotide triphosphates comprise dCTP, dGTP, dATP, and dTTP.
11. The method of claim 1 wherein the reaction mixture further comprises one or more divalent metal ions present at a concentration of about 5 mM.
12. The method of claim 11 wherein the divalent metal ions are magnesium and/or manganese present at a concentration of about 5 mM.
13. The method of claim 1 wherein the capture moiety is a hapten.
14. The method of claim 1 wherein the DNA primer comprises a capture moiety.
15. The method of claim 1 wherein the RNA template comprises a capture moiety.
16. The method of claim 1 wherein the detectable moiety is incorporated into the extended primer under conditions suitable to preserve the signal of the detectable moiety.
17. The method of claim 16 wherein the detectable moiety is an acridinium moiety, and the detectable signal is the emission of light.

18. The method of claim 5 wherein the deoxynucleoside triphosphates labeled with the acridinium moiety has the formula:



wherein:

TP is a triphosphate group attached to the 5' position of the sugar;

sugar is a sugar moiety;

Px is a purine, pyrimidine, or 7-deazapurine, wherein Px is attached to the 1' position of the sugar moiety through the N1 position when Px is a pyrimidine or through the N9 position when Px is a purine or a 7-deazapurine;

L is a linear or branched hydrocarbylene or heterocarbylene linker of at least one carbon atom, wherein L is covalently attached to Acr at one end of L, and at another end to Px through position C5 or C6 of Px when Px is a pyrimidine, or through position C8 of Px when Px is a purine, or through position C7 or C8 of Px when Px is a 7-deazapurine;

Acr is an acridinium moiety; and

the detectable signal is the emission of light.

19. The method of claim 18 wherein the linker, L, is a linear hydrocarbylene or heterocarbylene linker of at least one carbon atom.

20. The method of claim 18 wherein the linker, L, is a linear alkenylene or heteroalkenylene linker containing at least 3 carbon atoms.

21. The method of claim 19 wherein the linker L is $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$.

22. The method of claim 19 wherein the linker L is $-\text{CH}=\text{CH}-\text{CH}_2-\text{NH}-$.

23. The method of claim 18 wherein the acridinium moiety is stable under conditions of reverse transcription, and is detectable after incorporation into the extended primer by the emission of light.

24. The method of claim 1 wherein:
the deoxynucleotide triphosphates are labeled with a detectable moiety and with a capture moiety.

25. A molecular structure comprising an RNA template hybridized to an extending DNA primer, which comprises a deoxynucleotide linked to an acridinium moiety.

26. The composition of claim 25 wherein the RNA template further comprises a capture moiety.

27. The composition of claim 25 wherein the DNA molecule further comprises a capture moiety.

28. A kit comprising:
an RNA template;
a DNA primer complementary to a region of the RNA template and of length sufficient to form a stable template-primer hybrid molecule with the RNA template;
a deoxynucleotide triphosphate labeled with a detectable moiety.

29. The kit of claim 28 further comprising buffers for conducting a reverse transcriptase assay.

30. The kit of claim 29 wherein the buffers comprise a divalent metal ion at a concentration of about 5 mM.

31. The kit of claim 28 wherein the detectable moiety is an acridinium moiety.

32. The kit of claim 28 wherein the deoxynucleotide triphosphate further comprises a capture moiety.

33. A method of determining a sub-type of reverse transcriptase present or absent in a sample comprising:

contacting the sample with a binding molecule specific for a sub-type of reverse transcriptase;

contacting the sample with a reaction mixture comprising

an RNA template;

a DNA primer;

a deoxynucleotide triphosphate labeled with a detectable moiety; and

determining whether a molecular structure is generated comprising an extended DNA primer comprising said detectable moiety;

thereby determining whether the sub-type of reverse transcriptase is present or absent in the sample.

34. The method of claim 33 wherein one of said RNA template, DNA primer, and said deoxynucleotide triphosphate labeled with a detectable moiety comprises a capture moiety.

35. The method of claim 33 wherein the binding molecule is immobilized on a surface.

36. The method of claim 33 wherein the detectable moiety is an acridinium moiety.

37. A method of screening for anti-retroviral lead compounds, comprising:
contacting a compound to be tested for anti-retroviral activity with a sample of reverse transcriptase;

contacting the compound and reverse transcriptase with a reaction mixture comprising

an RNA template;
a DNA primer;
a deoxynucleotide triphosphate labeled with a detectable moiety; and
determining whether a molecular structure is generated comprising an extended DNA
primer comprising said detectable moiety; and
thereby screening the compound for anti-retroviral activity.

38. The method of claim 37 wherein one of said RNA template, DNA primer, deoxynucleotide triphosphate labeled with a detectable moiety, and deoxynucleotide triphosphate not labeled with a detectable moiety, comprises a capture moiety.

39. A method of detecting the presence of reverse transcriptase in a sample comprising:

contacting the sample with a reaction mixture comprising
an RNA template;
a DNA primer;
one or more deoxynucleotide triphosphates labeled with a detectable moiety;
wherein one of said RNA template or DNA primer is immobilized on a solid phase;
incubating said reaction mixture under conditions suitable to generate a molecular
structure comprising an extended primer comprising said detectable moiety when reverse
transcriptase is present in said sample; and
detecting reverse transcriptase in said sample.